Cell, Vol. 85, 997–1008, June 28, 1996, Copyright ©1996 by Cell Press

Differential Requirements for α4 Integrins during Fetal and Adult Hematopoiesis

Alicia G. Arroyo, Joy T. Yang, Helen Rayburn, and Richard O. Hynes
Howard Hughes Medical Institute
Center for Cancer Research and Department of Biology
Massachusetts Institute of Technology
Cambridge, Massachusetts 02139

Summary

Mice chimeric for the expression of α4 integrins were used to dissect the roles of these receptors in development and traffic of lymphoid and myeloid cells. During fetal life, T cell development is α4 independent, but after birth further production of T cells becomes α4 dependent. Precursors for both T and B cells require α4 integrins for normal development within the bone marrow. In contrast, monocytes and natural killer cells can develop normally without α4 integrins. Thus, there are lymphocyte-specific, developmentally regulated requirements for α4 integrins in hematopoiesis in the bone marrow. We also show that α4 integrins are essential for T cell homing to Peyer's patches, but not to other secondary lymphoid organs, including spleen, lymph nodes, and intestinal epithelium.

Introduction

Hematopoiesis, the process by which pluripotent precursor cells undergo differentiation into different blood cell lineages, requires diverse cell migration pathways and takes place in several organs (such as the yolk sac and fetal liver) during embryonic life and mainly in the bone marrow in the adult (for review see Morrison et al., 1995; Weissman, 1994). These migrations are believed to rely on specific adhesion receptors. The adhesive interactions of hematopoietic progenitors with bone marrow stromal cells and with the extracellular matrix (ECM) have also been shown to play a key role in their normal development and function (Gordon et al., 1987), but the details of these interactions remain unclear.

Integrins are a widespread family of heterodimeric (αβ) transmembrane glycoproteins that can function as cell–ECM or cell–cell adhesion receptors (for review see Hynes, 1992). α4 integrins are particularly interesting owing to their involvement in various developmental and physiological processes (Yang et al., 1995; see below). The two α4 integrins (α4β1 and α4β7) bind to an alternatively spliced segment of fibronectin (V25 or CS1) and to the counterreceptors, vascular cell adhesion molecule 1 (VCAM-1) and mucosal addressin cell adhesion molecule 1 (MAdCAM-1), and α4 integrins have recently been reported to recognize the α4 chain itself (Altevogt et al., 1995). α4 integrins have been suggested to play roles in hematopoiesis (Williams et al., 1991), lymphocyte homing (Holzmann and Weissman, 1989), recruitment of leukocytes to inflammatory foci (Yednock et al., 1992), and metastasis (Qian et al., 1994).

Previous reports have suggested an involvement of α4 integrins in hematopoiesis at several different levels, including the development of hematopoietic precursors, lymphoid and myeloid cells. α4 integrins are widely expressed on different cell types, including hematopoietic progenitors, lymphocytes, natural killer (NK) cells, monocytes, eosinophils, and basophils (for review see Hemler, 1990; Sánchez-Madrid and Corbí, 1992). Members of the β1 subfamily, including α4β1 and α5β1 and their counterreceptors, have been implicated in the attachment of hematopoietic precursors to bone marrow stromal cells and ECM in in vitro experiments (Kerst et al., 1993; Levesque et al., 1995; Miyake et al., 1991b, 1992; Simmons et al., 1992; Teixidó et al., 1992). In vivo experiments with antibodies have suggested a role for the common β1 chain of integrins in migration of precursors to the spleen (Williams et al., 1991), and no colonization of fetal liver by hematopoietic precursors is observed in the absence of β1 integrins (Hirsch et al., 1996). A role for the α4β1 receptor in the attachment and homing of hematopoietic progenitors to the bone marrow in primates and mice has also been proposed (Papayannopoulou and Nakamoto, 1993; Papayannopoulou et al., 1995).

A stroma-dependent phase has been described in the first stages of B cell development in the bone marrow and in the fetal liver during embryonic life (Gisler et al., 1987; Hardy et al., 1991; Strasser et al., 1989). In vitro studies have suggested roles for α4β1 in murine B cell differentiation (Miyake et al., 1991a) and in terminal differentiation of mature B cells from the bone marrow (Roldán et al., 1992). Concerning T cell development, T cell precursors migrate from the yolk sac and fetal liver to the thymus, where they develop. After birth, the bone marrow supplies the thymus with a constant input of precursors for normal maintenance of the T lymphocyte population (Jotereau et al., 1987; Scollay et al., 1986). Previous reports have shown that the expression of α4 integrins is differentially regulated in developing thymocytes, and a fibronectin receptor has been implicated in maturation of double-negative cells in the thymus in vitro (Salomon et al., 1994; Sawada et al., 1992; Utsumi et al., 1991). It has also been reported that myelopoiesis can be retarded in vitro by anti-α4 antibodies (Miyake et al., 1991a).

α4 integrins have also been implicated in regulation of leukocyte traffic. Studies with antibodies have suggested that the α4β7 receptor plays an important role during the migration of lymphocytes to mucosal-associated lymphoid tissues and intestine by recognizing its counterreceptor MAdCAM-1 (Hamann et al., 1994; Holzmann and Weissman, 1989) and in the recruitment of leukocytes to inflammatory foci (Yednock et al., 1992).

The studies reviewed above clearly suggest that α4 integrins could play important roles in the development and functions of lymphoid and myeloid cells. However, the majority of the results concern in vitro systems, and the functions of these integrins, particularly during development in vivo, remain poorly defined. To study the in vivo roles of the adhesion receptors α4β1 and α4β7, we knocked out the gene encoding the α4 chain in mice.

That mutation is embryonic lethal (Yang et al., 1995), so for the analysis of hematopoietic development we generated somatic chimeric mice by injecting $\alpha4^{-/-}$ embryonic stem (ES) cells into $\alpha4^{+/+}$ blastocysts. In this paper, we report the use of such chimeras to define the roles of $\alpha4$ integrins in lymphopoiesis, myelopoiesis, and lymphocyte traffic.

Results

A Switch in the Requirements for $\alpha4$ Integrins during T Cell Development

The in vivo role of the $\alpha4$ integrins in hematopoietic differentiation was studied by generation of $\alpha4$ null and $\alpha4$ control chimeric mice by injection of $\alpha4^{-/-}$ or $\alpha4^{+/+}$ ES cells into either C57BL or RAG-1- or RAG-2-deficient blastocysts. These chimeras will be referred to as $\alpha4$ null or $\alpha4$ control/C57BL or RAG-1 or RAG-2 chimeras. Since RAG-1- and RAG-2-deficient mice do not develop any mature lymphocytes, RAG-1 and RAG-2 chimeras are particularly good models to evaluate the involvement of a specific gene in lymphocyte development (Chen et al., 1993; Mombaerts et al., 1992; Shinkai et al., 1992).

Lymphocytes from different organs were analyzed by flow cytometry using the marker Ly-9.1, which specifically stains the lymphocytes derived from the ES cells (129Sv strain) and not those derived from the blastocyst (C57BL strain) (Ledbetter et al., 1979). Using this approach, normal percentages of ES-derived T cells (CD3/Ly-9.1 positive) were observed in the peripheral blood and spleen of adult $\alpha4$ null chimeric mice (>4 weeks old) as compared with control chimeras in either C57BL or RAG-1 (Figures 1A and 1B). These T cells were confirmed to be $\alpha4$ null by flow cytometry and polymerase chain reaction (PCR) analysis (data not shown). The CD4/CD8 ratio and T cell receptor $\alpha\beta$ (TCR$\alpha\beta$) or TCR$\gamma\delta$ expression displayed by these $\alpha4$ null T cells were normal, and they appeared to respond properly upon activation with different stimuli as assessed by expression of activation markers as well as proliferation rate (data not shown). Thus, development of various T cell subsets can proceed in the complete absence of $\alpha4$ integrins on the surfaces of these cells and their precursors.

Interestingly, when the thymi from $\alpha4$ null chimeric mice were analyzed, we observed a gradual decrease in $\alpha4$ null thymocytes after the birth of the animals. Thus, in $\alpha4$ null/RAG-1 or $\alpha4$ null/RAG-2 chimeric mice older than 3 weeks, very few CD4/CD8 double-positive cells remained, the thymus became progressively atrophic, and after 1 month only double-negative cells derived from the RAG-$1^{-/-}$ or RAG-$2^{-/-}$ blastocysts were observed compared with control mice, as assessed by flow cytometry (Figure 2) and histological analysis (Figures 3A and 3B). Since the wave of bone marrow–derived precursors is expected after birth, these data suggest that, in the absence of $\alpha4$ integrins, there is a defect in the input of T cell precursors from the bone marrow to the thymus.

To elucidate further the step of blockade (before, during, or after the bone marrow stage), reconstitution experiments were performed. Bone marrow from the $\alpha4$ null/RAG-1 chimeras injected into the blood of sublethally irradiated RAG-$1^{-/-}$ recipients was able to reconstitute the peripheral T cell population in the short term

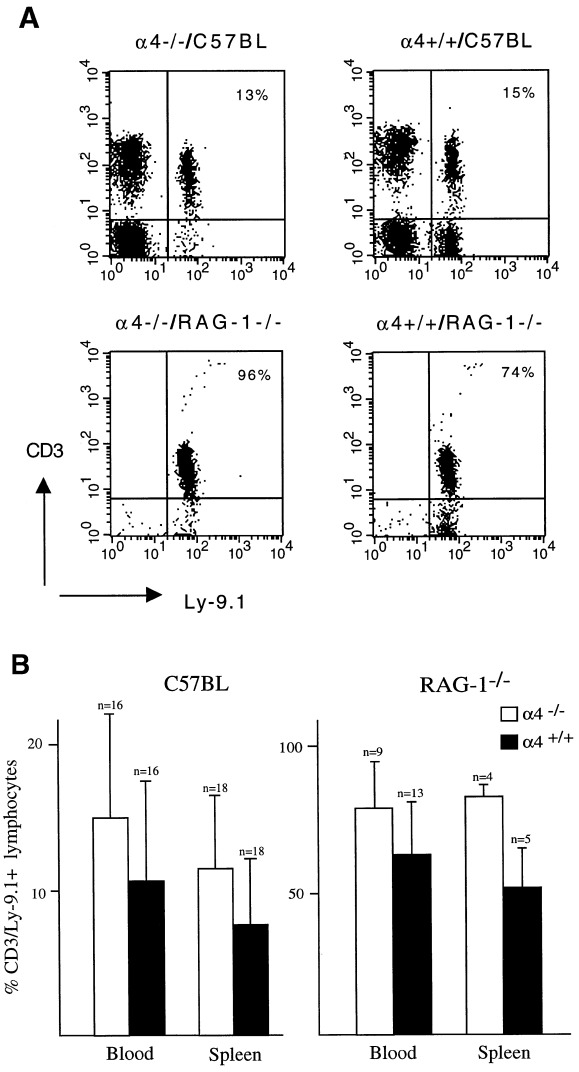

Figure 1. T Lymphocytes Lacking $\alpha4$ Integrins Can Develop Normally

(A) Blood from $\alpha4$ null and control chimeric mice was stained with anti-CD3 and anti-Ly-9.1 monoclonal antibodies, and lymphocytes were analyzed by flow cytometry. Note that the ES-derived T cell population (Ly-9.1$^+$/CD3$^+$) is similar in both null and control chimeras. The Ly-9.1$^+$/CD3$^-$ population present in $\alpha4$ control chimeras are B cells not seen in $\alpha4$ null chimeras (see text). This leads to the lower total percentages of Ly-9.1$^+$/CD3$^+$ cells in control chimeras.
(B) Lymphocytes from blood or spleen of chimeric mice (>30% coat color chimerism in C57BL or RAG-1) were stained and analyzed by flow cytometry as in (A). Mean and standard deviation are shown. Again, note that the percentages of T cells derived from $\alpha4^{+/+}$ ES cells are lower because in this case they also contribute to the B cell population (see text).

(Figure 4). This result means that the bone marrow contains $\alpha4$ null precursors able to migrate and differentiate in the thymus. No long-term reconstitution was observed, but secondary bone marrow transplantation from the $\alpha4$ null reconstituted mice into nonirradiated RAG-$1^{-/-}$ recipients again gave rise to peripheral T lymphocytes, showing, first, that proper migration of precursors into the bone marrow can take place in the absence of $\alpha4$ integrins but, second, that development of $\alpha4$ null

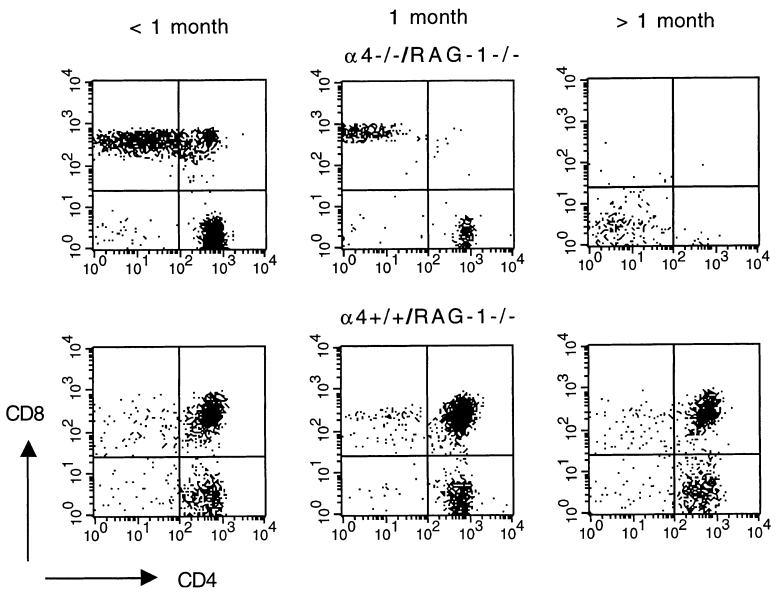

Figure 2. T Cell Development Is Blocked in the Thymi of α4 Null Chimeric Mice Older than 3 Weeks

Thymi from α4 null/RAG-1 and control/RAG-1 chimeric mice of different ages were analyzed for the expression of CD4 and CD8. Note that, while 10^4 cells were analyzed for control chimeras and for null chimeras less than 1 month old, the thymi of older chimeras are atrophic and fewer cells were analyzed (less than 500 cells). The important point is that the ratio of the different populations (double negative, double positive, and single positive) changes during the life of the null chimeras but does not vary in the control animals. After 1 month, only double-negative cells remain in the thymi of the null chimeras, and they are derived from the RAG-$1^{-/-}$ blastocysts. The number of thymocytes was on average 3×10^6 in null versus 100×10^6 in control chimeric mice older than 1 month.

T cell precursors to the point at which they can exit the bone marrow and migrate to the thymus does not occur (data not shown). Altogether, these results point to a problem within the bone marrow environment rather than in the seeding of the bone marrow with precursors or in the migration of T cell precursors into, or differentiation within, the thymus during adult life in the α4 null chimeras.

These data clearly show that α4 integrins are not essential for the in vivo development of mature T cells in the thymus during embryonic life, although they play a key role in the maintenance of normal T cell homeostasis during adult life, probably by regulating the normal development of T cell precursors in the adult bone marrow.

α4 Null T Lymphocytes Cannot Migrate to Peyer's Patches

The specific localization of α4 null T lymphocytes (homing) was studied by the analysis of different secondary lymphoid organs from chimeric mice. As shown in Figure 5, very few α4 null T lymphocytes were detected in Peyer's patches (gut mucosal-associated lymphoid tissue) of α4 null/C57BL mice, and, remarkably, no Peyer's patches could be found in the α4 null/RAG-1 chimeras. The levels of other adhesion receptors potentially involved in that migration pathway, such as L-selectin and CD44 in the α4 null T cells, were comparable with controls (data not shown). In contrast, percentages of α4 null T lymphocytes comparable with those seen in control animals were observed in the lymph nodes (mesenteric and inguinal) as well as within the intraepithelial compartment of the intestine of α4 null chimeras (Figures 5A and 5B). The sizes of the lymph nodes were similar in α4 null/RAG-1 and control/RAG-1 chimeric mice, and the histological analysis of these organs demonstrated a similar structure, although no germinal centers (B cell areas) were apparent in the α4 null chimeras (data not shown; see below).

These data clearly demonstrate that α4 integrins are essential for the in vivo homing of T lymphocytes to Peyer's patches but do not play an important role in the migration to lymph nodes and intestinal epithelium.

B Cell Maturation Is Severely Compromised in α4 Null Chimeric Mice

As shown in Figure 6, the number of B lymphocytes derived from α4 null ES cells, assessed by the double expression of CD45R(B220) and Ly-9.1 markers, was greatly decreased in the blood of α4 null chimeric mice compared with a normal ES-derived B cell chimeric population in control animals both in C57BL and RAG-1 chimeras. The percentage of α4 null B cells present in C57BL chimeric mice was a little higher in animals younger than 3 weeks (Figure 6B), suggesting that some B cell development could have taken place during embryonic life in the absence of α4 integrins. The reduced B cell population was mature, since it expressed surface immunoglobulin M (IgM), and the levels of serum IgM in the null chimeras were comparable with controls (data not shown). Similar results were obtained when other lymphoid organs such as spleen and lymph nodes were analyzed (Figure 6A; data not shown). In fact, spleens from α4 null/RAG-1 chimeric mice were smaller than controls, and histology revealed an altered architecture showing a marked defect in the B cell–specific areas as compared with control animals (data not shown).

A different subset of B cells (B-1 cells) present mainly in the peritoneal cavity in the adult and expressing specific markers such as CD5 was also analyzed (Kantor and Herzenberg, 1993). Very few CD45R/$CD5^+$ cells (4.2% in α4 null/RAG-1 chimeras versus 22.3% in controls [n = 4]) were present in the peritoneal cavity of α4 null chimeras (Figure 6A), suggesting a defect in B-1 cell migration to, or proliferation or survival in, the peritoneal cavity in the absence of α4 integrins.

The bone marrow obtained from the different chimeric mice was also analyzed. A greatly reduced number of $CD45R^+$ cells was found in the bone marrow of α4 null chimeric mice compared with controls (Figures 7A and 7D). Since the RAG-$1^{-/-}$ mice are on a mixed genetic

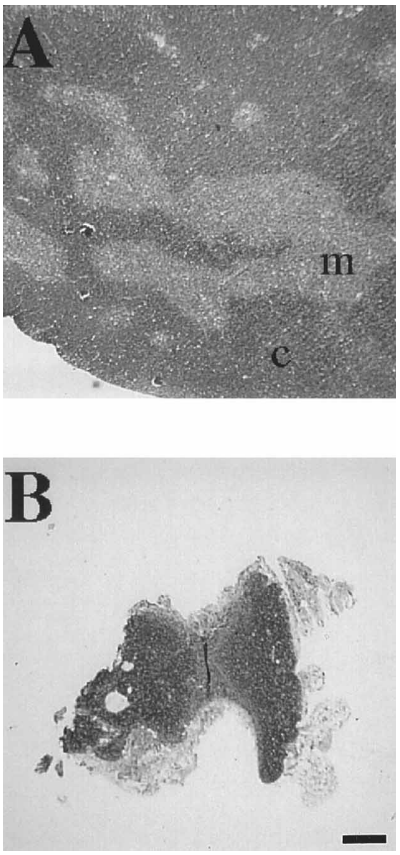

Figure 3. Thymi from α4 Null/RAG-1 or RAG-2 Chimeric Mice Become Progressively Atrophic

Histological analysis of thymi from α4 null/RAG-2 chimeric mice was performed at different timepoints (1 week in [A] and 2 months in [B]). Note that in α4 null chimeras older than 2 months (B) the normal structure of the thymus (A) with cortical (c) and medullary (m) areas is not observed. Moreover, the thymi from those null chimeras are much smaller and atrophic. The appearance of thymi from α4 control chimeras was similar to that shown in (A) at any timepoint analyzed (data not shown). Scale bar is 200 μm.

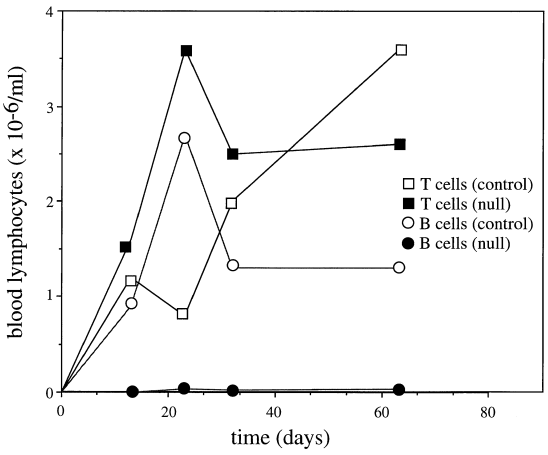

Figure 4. Bone Marrow from α4 Null Chimeras Is Able to Reconstitute the Peripheral T Cell Compartment of Sublethally Irradiated Recipients

Bone marrow cells from α4 null/RAG-1 or control/RAG-1 chimeric mice were injected into irradiated RAG-$1^{-/-}$ recipients. The presence of reconstituted T and B cell lineages was determined at different timepoints by flow cytometry analysis of blood lymphocytes stained for CD3 and CD45R expression, respectively. Each point represents the mean of four mice transplanted. Standard deviation was 10%–15% for each point. Note that α4 null bone marrow is able to reconstitute a normal number of T lymphocytes in the periphery, although it fails to reconstitute the B cell population (see text for further details about the B cell defect). Control bone marrow reconstitutes both populations. Thymi from α4 null reconstituted mice were normal 1 month after injection, but became atrophic after 2 months (data not shown). PBS-injected mice did not show any T or B blood lymphocytes (data not shown).

background, we could not use the Ly-9.1 marker to distinguish whether those few B cells were immature pro-B cells normally present in the RAG-1-deficient mice or whether they derived from the $\alpha4^{-/-}$ ES cells. However, most of those $CD45R^{+}$ cells in the α4 null/RAG-1 chimeras were positive for α4 expression, demonstrating that they derived from the RAG-1 blastocyst. Moreover, a few α4 null/RAG-2 chimeric mice analyzed by specific markers showed almost no α4 null/$CD45R^{+}$ cells (data not shown). These results show that, in the absence of α4 integrins, B cell development is blocked at an earlier stage than that observed in RAG-1- or RAG-2-deficient mice, i.e., before pro-B cell stage. To investigate this point further, we also stained this $CD45R^{+}$ population for markers differentially expressed along the B cell development pathway, including CD43 and surface IgM. In fact, most were immature pro-B cells ($CD45R^{dull}$/$CD43^{+}$) derived mainly from the RAG-$1^{-/-}$ blastocysts (Figures 7B and 7D). Pre-B cells ($CD45R^{high}$/$CD43^{-}$) and mature B cells ($CD45R^{+}$/IgM^{+}) were almost absent in α4 null chimeras as compared with the control mice (Figures 7B–7D), demonstrating a severe failure in in vivo B cell development in the absence of α4 integrins. Furthermore, bone marrow from α4 null chimeras failed to reconstitute the B cell compartment of irradiated recipients carrying wild-type bone marrow stroma (see Figure 4), confirming the in vivo inability of B cell precursors to develop properly in the bone marrow when they do not express α4 integrins after birth. In contrast, preliminary data showing the ability of fetal liver from α4 null chimeras to reconstitute a normal B cell population suggest that B cell development from fetal precursors can take place without α4 integrins (data not shown).

Altogether, these data clearly demonstrate that the α4 integrin adhesion receptors are essential for normal in vivo B lymphocyte differentiation after birth and that a severe defect at the B cell precursor level occurs in the adult bone marrow in the absence of α4 integrin expression.

Monocytes and NK Cells Can Undergo Complete Development without Expressing α4 Integrins

The presence of monocytes derived from α4 null precursors was assessed by testing for the double expression of the monocyte marker Mac-1 (CD11b/CD18) as well as the α4 integrin chain in peripheral blood. A subset of Mac-1^{+}/α4-negative cells was found in the peripheral blood of α4 null chimeric mice, while no α4-negative cells within the monocyte Mac-1^{+} population could be detected in the control animals (Figure 8). The double

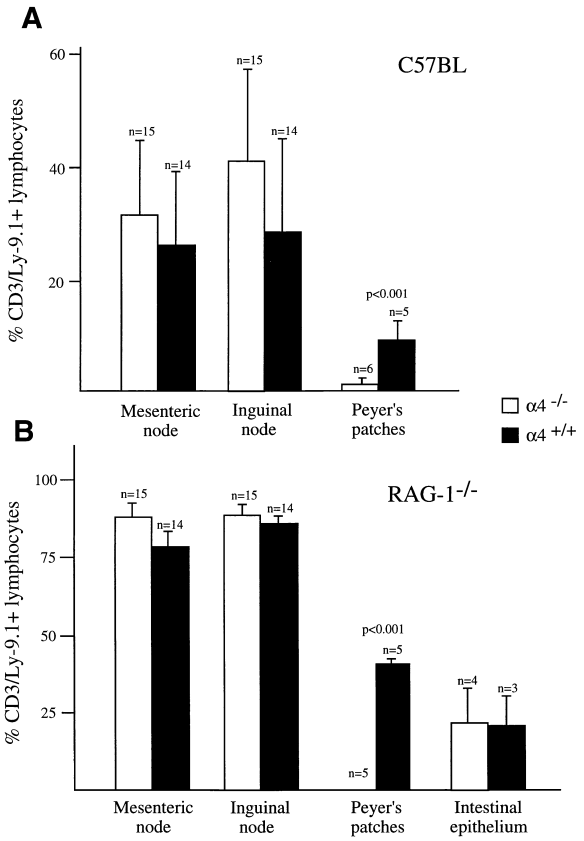

Figure 5. α4 Null T Lymphocytes Are Unable to Migrate to Peyer's Patches

Lymphocytes from different lymphoid tissues and intestine of the different chimeras were stained for CD3 and Ly-9.1 expression and analyzed by flow cytometry. Mean and standard deviation are shown. Note that no α4 null T cells are found in Peyer's patches. The differences in lymphocyte populations from mesenteric and inguinal nodes and intestine are not significant.

expression of CD44 (to stain monoblastic and monocytic cells) and α4 was also analyzed in a monocytic population (gated by size and complexity) from the bone marrow of these animals. A proportion of $CD44^+$/α4-negative cells was present in α4 null chimeric mice, but no α4-negative cells were found within the monocytic $CD44^+$ population in control animals (Figure 8). There was no correlation between the percentage of α4 null monocytes and the age of the mice, suggesting no defect in monocytic precursors lacking α4 integrins during adult life.

NK cell development was also investigated. Specific markers for this lineage such as NK-1.1 are not expressed in NK cells from the 129Sv strain. Therefore, we used the $CD8\alpha^+/CD3^-$ phenotype in a population gated by size and complexity to identify NK cells in the blood of adult chimeric mice (>9 months old). A larger population of α4-negative NK cells was present in the α4 null/C57BL chimeras compared with controls (21.6% in α4 null versus 7.1% in α4 control chimeras [n = 8]).

These data demonstrate that monocytes and NK cells do not require α4 integrin receptors to become mature cells able to circulate in peripheral blood.

Discussion

Here we have addressed several questions about the roles of α4 integrin adhesion receptors during in vivo hematopoiesis. We find a lymphocyte-specific defect in the absence of α4 integrins, since monocyte and NK cell development are not detectably affected. Furthermore, we show that α4 integrins are not essential in the migration and cellular interactions that occur during embryonic lymphocyte development, but, interestingly, we detect a developmental switch in the requirements for α4 integrins, since after birth no further T or B cell development occurs in the bone marrow in the absence of α4 integrins. Moreover, α4 integrins are completely required for lymphocyte homing to Peyer's patches (Figure 9).

T Cell Development

T cell precursors originate in the yolk sac and fetal liver and migrate into the thymus to differentiate into single-positive $CD4^+$ or $CD8^+$ T lymphocytes. After birth, the bone marrow constitutes the only organ where the T cell precursors proliferate to supply the thymus with a constant input of progenitors for normal maintenance of the T cell population (Jotereau et al., 1987; Scollay et al., 1986). In α4 null/RAG-1 chimeras where no $\alpha4^{+/+}$ lymphocytes are present, phenotypically and functionally normal T lymphocytes can develop without any α4 integrin receptors (Figure 1). It is possible that subtle functional defects could occur in these α4 null T cells, since they are developed from embryonic precursors and a more restricted TCR repertoire with fewer N-nucleotide insertions would be expected (Lafaille et al., 1989). Thus, α4 integrins are not essential for T cell precursors to migrate to the thymus or for T cell precursors to interact with the thymic epithelium or ECM to become mature T lymphocytes. While α6β1 plays a role in pro-T cell homing to the thymus (Ruiz et al., 1995), and E-cadherin and likely CD81(TAPA-1) function in thymocyte–thymic epithelium interactions (Boismenu et al., 1996; Lee et al., 1994), α4 integrins do not appear to play an essential role in any of these processes.

However, it is important to note the switch in the requirements for α4 integrins during T cell development. While embryonic precursor interactions with the microenvironment do not require these receptors, after birth α4 integrins become essential for further T cell development, since no α4 null thymocytes were detected in the thymus of chimeric mice older than 3 weeks (Figures 2 and 3). These data suggest several different points at which T cell precursor development could be blocked after birth: migration of precursors from the fetal liver to the bone marrow, defective differentiation in the bone marrow, exit from the bone marrow, or migration to or differentiation of the precursors in the thymus. To determine which of these steps is α4 integrin dependent, we performed reconstitution experiments. A short-term reconstitution of the T cell population was obtained when bone marrow from α4 null chimeras was injected into the blood of irradiated or nonirradiated recipients (Figure 4; data not shown). These data rule out a failure of precursors to migrate to or differentiate in the thymus,

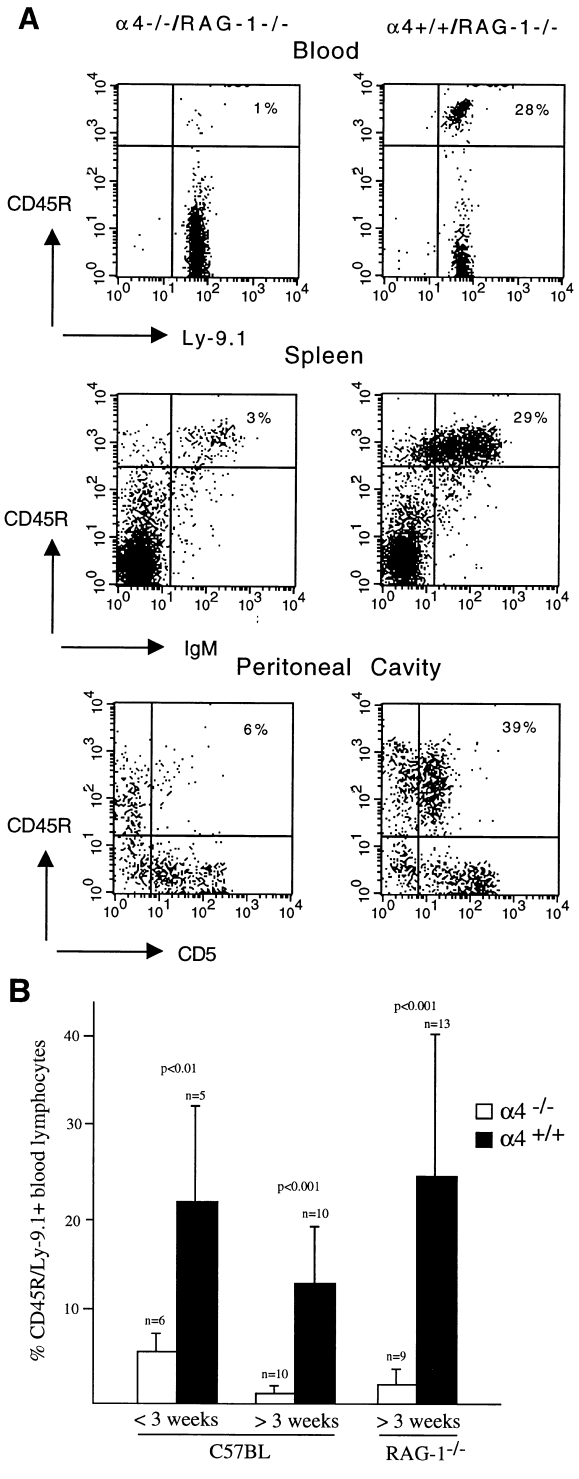

Figure 6. B Cell Development Is Severely Compromised in α4 Null Chimeric Mice

(A) Lymphocytes from blood, spleen, and peritoneal cavity from α4 null/RAG-1 or control/RAG-1 chimeric mice were analyzed for the expression of different B cell markers and Ly-9.1. Note that very few conventional B cells and B-1 cells are present in blood and spleen and in the peritoneal cavity, respectively, of α4 null chimeras as compared with controls.

(B) C57BL or RAG-1 chimeric mice (>30% of coat color chimerism) were analyzed. Lymphocytes from blood were stained and analyzed by flow cytometry. The mean and standard deviation are shown. Greatly reduced numbers of CD45R$^+$ B lymphocytes were observed in the α4 null chimeras. Similar percentages of IgM$^+$ cells were found within the B cell population of the different chimeras, and similar results were obtained when lymphocytes from spleen were analyzed (data not shown). The number of cells in the spleen was on average 67 × 10^6 in null/RAG-1 versus 122 × 10^6 in control/RAG-1 chimeric mice.

as well as a defect in migration of precursors from the fetal liver to the bone marrow, since T cell precursor activity is present in the bone marrow from α4 null chimeric mice. We have not yet elucidated the exact nature of the defect, but it seems to be at the bone marrow level. A defect in the exit per se of T cell progenitors from the bone marrow in the absence of α4 integrins could be possible; however, in this case an accumulation of precursors would be expected but is not observed. It is more likely that the α4 null T cell progenitors cannot migrate from the bone marrow either because they do not survive long enough or because they fail to reach the maturation state required to exit the bone marrow. Altogether, the data point to a defect in the specific interactions between progenitors for T cells and the bone marrow in the absence of α4 integrins, as will be discussed later.

B Cell Development

It was previously reported that there is a stroma-dependent phase in the first stages of B cell development in the bone marrow and in the fetal liver at the embryonic stage (Gisler et al., 1987; Hardy et al., 1991; Strasser et al., 1989). Although some in vitro studies suggested a role for α4 integrins during B lymphopoiesis (Miyake et al., 1991a), little was known about the in vivo functions of these receptors in this process. In this report, we show that α4 integrins are essential for complete maturation of B cells in vivo, since very few B cells are generated and development is largely blocked before the pro-B cell stage in the absence of α4 integrins (Figures 6 and 7). These receptors may mediate key interactions between the progenitors and the bone marrow stromal cells or the ECM (or both). These interactions might be important only in the bone marrow during adult life, as the few α4 null B cells detectable could have been developed during embryonic life from fetal liver precursors. This idea is supported by the higher number of B cells found in α4 null chimeras younger than 3 weeks and the presence of B cell reconstitution in contrast with the absence of such reconstitution in fetal liver versus bone marrow transplant experiments (Figure 4; data not shown).

Why are these α4-mediated interactions essential for B cell development in the bone marrow? There are several possibilities. First, it is possible that the progenitors require an active signal for survival and that this signal could be provided by α4 integrin–mediated interactions with the stromal cells or the ECM. Integrin-mediated interactions seem to be essential for cell survival (Boudreau et al., 1995; Ruoslahti and Reed, 1994). In this regard, a role for α4 integrin–VCAM-1 interactions in the survival of activated B cells in the germinal centers has been reported (Koopman et al., 1994), and it has also

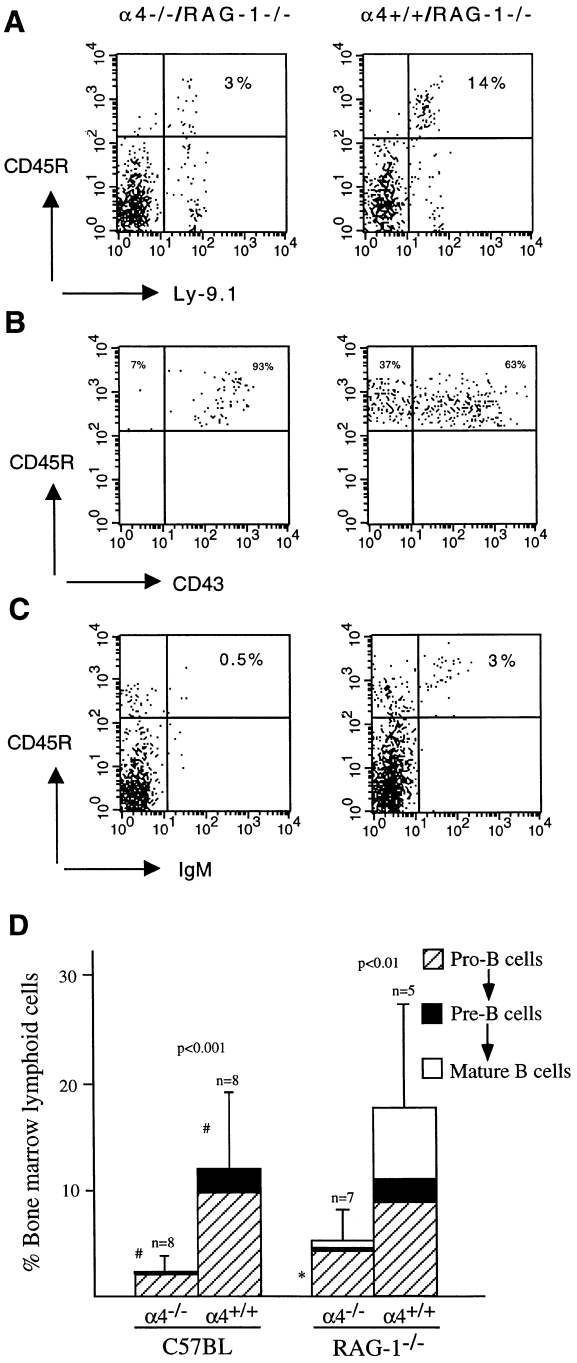

Figure 7. B Cell Precursors Fail to Mature in the Bone Marrow when They Lack α4 Integrins

(A–C) Bone marrow from α4 null/RAG-1 or control/RAG-1 chimeric mice was obtained and analyzed for the expression of markers differentially expressed during B cell development. Note that very few CD45R$^+$ cells are Ly-9.1$^+$ in the α4 null chimeras, and these are mostly pro-B cells (CD45R^{dull}/CD43$^+$) derived from the RAG-1$^{-/-}$ blastocyst. No mature B cells (CD45R$^+$/IgM$^+$) are present in the α4 null chimeras. In (A) and (C), total bone marrow populations are analyzed. In (B), gated populations positive for expression of Ly-9.1 and CD45R are analyzed.

(D) Cells from bone marrow of chimeric mice were obtained, stained for different markers, and analyzed by flow cytometry. The total number of bone marrow cells from two femora was on average 84 × 10^6 in α4 null/RAG-1 versus 69 × 10^6 in control/RAG-1 chimeric mice. The percentages, with mean and standard deviation, of the different B cell populations are shown. Note that only pro-B cells (CD45R^{dull}/CD43$^+$) and almost no pre-B (CD45R^{high}/CD43$^-$) or mature B cells (CD45R$^+$/IgM$^+$) are detected in the α4 null chimeras. Number signs indicate that the percentage of mature B cells in C57BL chimeras was not determined. The asterisk indicates that most of these pro-B cells were derived from the RAG-1$^{-/-}$ blastocysts, since they are positive for α4 expression. This means that the real number of CD45R$^+$ cells derived from the α4 null ES cells is even lower. In fact, a few α4 null/RAG-2 chimeras analyzed with specific markers showed almost no α4 null/CD45R$^+$ cells.

been shown that bone marrow fibroblasts can rescue plasma cells from apoptosis (Merville et al., 1996). This interesting possibility deserves further investigation. A second possibility is that α4 integrin–ligand interactions can trigger an active signal for synthesis of cytokines necessary in B cell development such as interleukin-7 (IL-7). It is known that progenitor–stroma interactions can induce the secretion of soluble growth factors (Sudo et al., 1989), although a recent report has shown that the stimulation of tyrosine kinases and IL-6 production on human bone marrow stromal cells induced by contact with B lymphocytes seems to be α4 independent (Jarvis and LeBien, 1995). A third possibility is that α4-mediated interactions are necessary to trigger directly intracellular signals essential for B cell development. In fact, mice deficient for several genes involved in signal transduction, such as Syk and Jak3 tyrosine kinase genes, display a similar phenotype, with B cell development greatly impaired (Cheng et al., 1995; Nosaka et al., 1995; Russell et al., 1995; Thomis et al., 1995; Turner et al., 1995).

Finally, the B-1 cell lineage defect observed in the absence of α4 integrins could be due to α4-mediated interactions playing a role in the migration of these cells into, or their survival or proliferation within, the peritoneal cavity. In fact, a role for peritoneal stromal cells in the survival of surface IgM$^+$ peritoneal B cells has been reported (Hardin et al., 1995). Another possibility is that the bone marrow may play a role in the maintenance of the B-1 cell population in the peritoneum, and since there is a defect in early B cell precursor development, the B-1 lineage would also be affected.

Bone Marrow Lymphopoiesis

Altogether, the data reported here concerning T and B cell development suggest that there may be a similar or common defect in both lineages and a switch in the dependence on α4 integrins during lymphopoiesis. Thus, lymphopoiesis seems to be independent of α4 integrins during embryonic life, but starts to be dependent on α4 integrins when progenitors move to the bone marrow after birth. It is possible that, in the absence of α4 integrins, fewer progenitor cells migrate to the bone marrow, as a recent report has suggested (Papayannopoulou et al., 1995). However, even a decreased number of progenitors should be able to reconstitute a normal lymphoid population, if the cells proliferate properly. This switch in the requirements for α4 integrins indicates that there is a strict regulation in time or space (or both) of α4-mediated functions during lymphocyte development. This switch could occur within a single lineage of

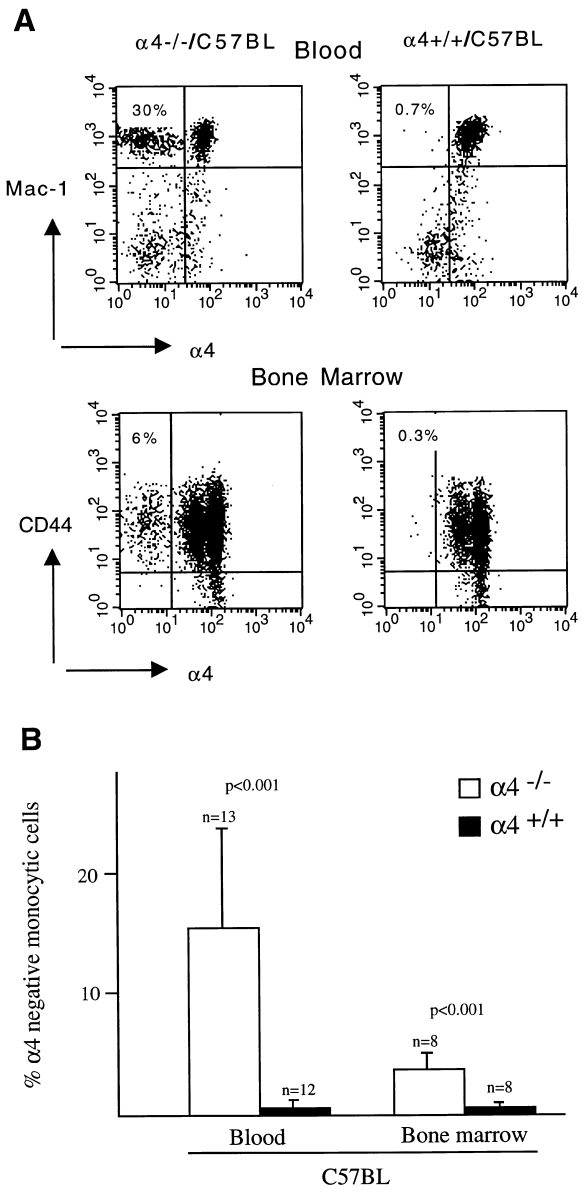

Figure 8. Monocytes Develop Normally without Expressing α4 Integrins

(A) Monocytes from blood and monocyte precursors from bone marrow were analyzed for the expression of specific markers and α4 integrins. Note that a subset of the monocytic population in both blood and bone marrow is negative for α4 expression in α4 null chimeras, in contrast with the absence of α4-negative cells in control animals.

(B) Cells from blood and bone marrow of chimeric mice were stained and analyzed for α4 integrins as well as Mac-1 or CD44 expression. Mean and standard deviation are shown. Note that a population of α4-negative monocytes is present in α4 null chimeras but absent in controls.

precursors as they develop or between separate fetal and adult lineages. It might be possible that α4 integrins are not expressed by fetal hematopoietic precursors, although expression of these receptors in the yolk sac and fetal liver of murine embryos has been shown (Sheppard et al., 1994), or that other receptors play the main role in attachment of lymphoid precursors to the stroma during embryonic development.

The consequences of such a common defect in T and B cell precursor functions in the adult in the absence of α4 integrins would be very different because of the different kinetics that govern T and B cell development. Since T cell development takes place in the thymus mainly during fetal life and, as shown in this report, α4 seems not to play an essential role in hematopoietic fetal migration and differentiation pathways, it is possible to find relatively normal percentages of α4 null T cells in the adult mice analyzed. However, because few B cells develop before birth and the large expansion of B cell development occurs normally in the first few months after birth, we detect in this case a severe defect in the B cell population lacking α4 integrins. The defect might be occurring at a point between the pluripotent stem cell and the stage of a common precursor for T and B cells in the bone marrow, since only the lymphoid lineages (T and B) and neither monocytes nor NK cells are affected in the adult chimeric mice. Another possibility is that committed T and B cell progenitors have the same α4 integrin requirements and differ in this respect from NK cell and myeloid precursors. Whichever is the case, it is clear that α4 integrin–mediated interactions between early T and B cell progenitors and the stromal environment in the bone marrow are regulating key events required for normal development of such precursors. If such a critical point is skipped in embryonic development, for instance because other adhesion receptors are involved or the microenvironment or the requirements for signaling are different, then the precursors differentiate normally. In fact, it is well known that there are many switches concerning differentially regulated gene expression and the ability of precursors to differentiate, for example, during the development of the immune system in embryonic versus adult life (Li et al., 1993; for review see Weissman, 1994).

As pointed out before, α4-mediated interactions might be regulating the proliferation, survival, differentiation, signal transduction pathways, or synthesis of soluble growth factors required for normal development of lymphoid precursors. Interestingly, mice deficient for genes involved in regulation of cell death and cell growth (*bcl-2* and c-*abl*, respectively) show normal development of the immune system at birth, but defects start to appear after a few weeks, pointing to different regulation and requirements for these genes during embryonic and adult life, as is the case for α4-mediated interactions (Nakayama et al., 1993; Schwartzberg et al., 1991; Tybulewicz et al., 1991; Veis et al., 1993). Mice deficient for another gene involved in inhibition of apoptosis, *bcl-x*, also show a defect in the immature hematopoietic precursors (Motoyama et al., 1995). It will be interesting to investigate further the lymphoid-specific signals likely mediated by α4 integrin interactions.

In summary, it was already known that bone marrow stromal cells support normal development of hematopoietic precursors (for review see Dorshkind, 1990; Kincade et al., 1989). In this report, we show that α4 integrins are key receptors mediating those interactions in vivo, since lymphoid development is completely blocked in the bone marrow at a very early stage in the absence

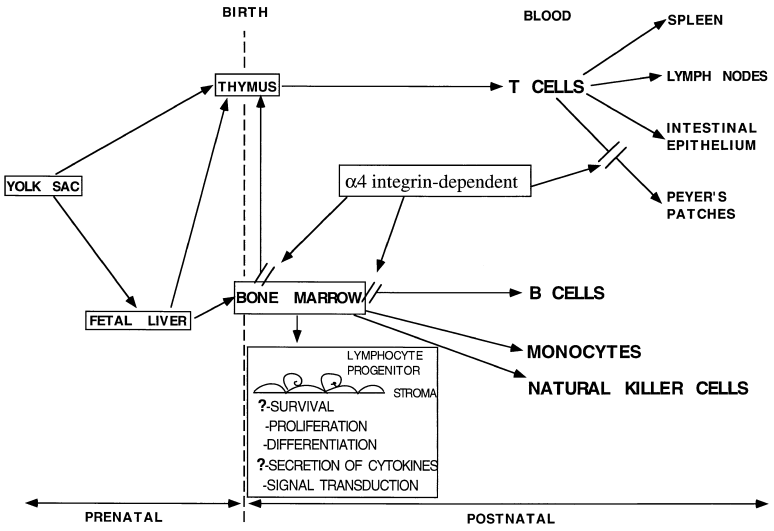

Figure 9. α4 Integrins Play Essential Roles during In Vivo Lymphoid Development and Traffic

Normal pathways of development and traffic of different leukocyte lineages before and after birth are shown. The analysis of α4 null chimeric mice has revealed important steps in which α4 integrins play essential roles. α4 integrins do not appear to play essential roles during embryonic lymphoid development (neither in migration pathways from the yolk sac to the fetal liver and to the thymus or bone marrow nor in the interactions of the progenitors with the microenvironment in these hematopoietic organs). After birth, α4 integrins become essential for interactions between T and B cell progenitors and the bone marrow stromal environment producing a complete blockade in B cell development and a cessation of the T cell development that occurred normally in the thymus during embryonic life. In the box are shown several different processes that these interactions might be regulating. α4 integrins are also essential for migration of T cells to Peyer's patches, but not to other lymphoid organs. In contrast, NK cells and monocytes lacking α4 integrins can develop normally without expressing α4 integrins. The number of B-1 cells is also greatly diminished in the absence of α4 integrins, although it is not yet clear at which step α4 integrins are required.

of α4 integrins. Regarding the specific integrins and counterreceptors likely involved in these essential α4 integrin–mediated interactions, it is worth mentioning that β7 knockout mice, recently obtained, do not show any defects in T or B cell development (Wagner et al., submitted), pointing to the recognition of fibronectin or VCAM-1 (or both) by the α4β1 heterodimer as being responsible for the defect observed at the bone marrow level in our α4 null chimeras.

Monocyte and NK Cell Development

The hematopoietic defect observed in the absence of α4 integrins seems to be lymphocyte specific, since no defect in monocyte or NK cell development could be detected when the precursors do not express α4 integrins (Figure 8). These data are somewhat in contrast with a report in which anti-α4 monoclonal antibodies were able to retard myelopoiesis in vitro (Miyake et al., 1991a). Nevertheless, our data indicate that monocytes do not need to express α4 integrins to develop normally, although a delay in their development would be difficult to detect. A role for a soluble factor secreted as a consequence of α4 integrin–mediated interactions of the wild-type monocytes present in the chimeric mice also cannot be ruled out at this point. The different requirements for α4 integrins between NK and T or B cell progenitors suggest that these lineages are in fact different and they separate early during ontogeny.

T Cell Homing

After maturation in the thymus, T lymphocytes exit to the peripheral blood and lymph where they recirculate and, by recognizing specific addressins, migrate or localize in the different secondary lymphoid organs (for review see Springer, 1994). A role for the α4 integrin subunit associated with the β7 chain and recognizing the ligand MAdCAM-1 in T lymphocyte migration to the mucosal-associated lymphoid tissue (Peyer's patches) and intestine has previously been reported, although other receptors such as L-selectin have also been suggested to play a role (Hamann et al., 1994). In the migration of T lymphocytes to peripheral nodes, other adhesion receptors have been implicated, including L-selectin and CD44. Here we show that in vivo α4 integrins are absolutely required for the migration of T lymphocytes to Peyer's patches (Figure 5), regardless of the presence of other adhesion receptors such as L-selectin. However, no decrease in migration of gut intraepithelial lymphocytes was detected in the absence of α4, suggesting that other receptors play the major role in that process. Interestingly, β7 knockout mice (Wagner et al., submitted) show a decreased but not zero number of lymphocytes in the Peyer's patches and intestine. Together with our data, this suggests that the α4β1 heterodimer may also play a minor role in homing to Peyer's patches and that the integrin αEβ7 plays the main role in migration to the intestinal epithelium.

In summary, this report provides insights into the in vivo roles of the two α4 integrin adhesion receptors during murine lymphopoiesis, monocyte development, and lymphocyte homing (Figure 9). Interestingly, α4-mediated events involved in lymphopoiesis are finely regulated in time or space (or both), since there is a switch in their requirements from prenatal to postnatal life. The data presented here reveal the importance of cellular adhesion events in regulating essential processes in lymphocyte differentiation and traffic.

Experimental Procedures

Chimeric Mice

Double knockout (i.e., homozygous null) α4 ES cells were obtained from heterozygous ES cell clones (Yang et al., 1995) by selection in high concentrations (1–1.5 mg/ml) of G418 (Mortensen et al., 1992). Chimeric mice were generated by injection of ES cells into blastocysts as previously described (George and Hynes, 1994). Other details of the generation and characterization of these $\alpha4^{-/-}$ ES cells

and chimeric mice will be reported elsewhere (J. T. Y. et al., unpublished data). Two independent α4 null clones (4D47 and 4D20), wild-type (clone D3), or heterozygous $\alpha4^{+/-}$ (clone 182) ES cells were plated on feeders 2 days before the injection in DME medium plus 20% FCS, 1× nonessential amino acids (GIBCO BRL), 0.1 mM β-mercaptoethanol, L-glutamine, and LIF (ESGRO from GIBCO BRL). The ES cells are derived from the 129Sv strain (agouti coat color). Blastocysts were isolated at 3.5 days postcoitum (dpc) from hormone-stimulated females (either C57BL strain or RAG-1- or RAG-2-deficient mice on mixed C57BL × 129Sv background, but with black coat color [a/a]). Approximately 15 ES cells (α4 null or wild type) were injected into each blastocyst, and the blastocysts were transferred into the uteri of pseudopregnant recipients 2.5 dpc. When the litters were born, the chimerism was estimated by coat color. In this report, we show the results obtained injecting D3 wild-type ES cells as controls, but similar data were obtained when heterozygous $\alpha4^{+/-}$ ES cells were used. Mice were kept in the Massachusetts Institute of Technology animal facility under standard conditions. RAG-1- and RAG-2-deficient mice were provided by Drs. J. Lafaille and S. Tonegawa and by J. Chen, respectively.

Flow Cytometry Analysis

The following monoclonal antibodies from Pharmingen were used for flow cytometry staining: PE-conjugated anti-mouse CD3ε (145-2C11), PE-conjugated anti-mouse CD45R/B220 (RA3-6B2), FITC-conjugated anti-mouse Ly-9.1 (Lgp100, 30C7), PE-conjugated anti-mouse CD4 (L3T4, RM4-5), CyC-conjugated anti-mouse CD8α (Ly-2, 53-6.7), FITC-conjugated anti-mouse CD5 (Ly-1, 53-7.3), biotinylated anti-mouse CD43 (leukosialin, S7), and CyC-conjugated streptavidin, PE-conjugated anti-mouse CD44 (Pgp-1, Ly-24 or H-CAM, IM7), and FITC- and PE-conjugated anti-mouse CD49d (integrin α4 chain, R1-2). PE-conjugated anti-mouse Mac1 (CD11b/CD18, Ly-40, M1/70) from Boehringer Mannheim was also used. Whole blood or single cell suspensions from different lymphoid organs obtained by routine techniques were incubated with purified anti-mouse CD32/CD16 (Pharmingen) to block binding via Fc receptors and with an appropriate dilution of the different antibodies at room temperature or 4°C, respectively. Red blood cells were lysed using lysis solution (Becton Dickinson). The samples were washed twice with PBS and resuspended in PBS. Dead cells in lymphoid organ samples were excluded by propidium iodide staining. The samples and the data were analyzed in a FACScan using CellQuest software (Becton Dickinson).

PCR Analysis

Lymphocytes from the spleens of α4 null/C57BL chimeras were sorted in a FACStar Plus (Becton Dickinson) cell sorter for the expression of the α4 chain and Ly-9.1 markers. Cells were lysed in a buffer containing 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 2.5 mM $MgCl_2$, 0.1 mg/ml gelatin, 0.45% NP-40, 0.45% Tween 20, and 2 mg/ml proteinase K. DNA was subjected to PCR using α4-specific primers as well as primers for the *neo*^{R} gene (Yang et al., 1995). Reactions were run as described by the manufacturer of Taq polymerase (Perkin-Elmer Cetus) under the following conditions: 72°C for 10 min and then 40 cycles of 94°C for 1 min, 55°C for 2 min, and 72°C for 4 min.

Histological Analysis

Tissue specimens were embedded in paraffin. Blocks were cut, and slides were processed and stained with hematoxylin–eosin by routine techniques. Slides were examined and photographed (Ektachrome 160T film; Eastman Kodak) with an Axiophot microscope (Carl Zeiss).

Reconstitution of Irradiated Mice

RAG-1-deficient mice (6–12 weeks old) were used as recipients for bone marrow transplantation experiments. Those mice were nonirradiated or sublethally irradiated (400 rads of γ-rays) 4 hr before the injection. Cells from bone marrow of α4 null or α4 control/RAG-1 chimeras were obtained and resuspended in sterile PBS. Approximately 10^7 cells were injected intravenously (retrorbitally) in each irradiated recipient. The presence of T and B lymphocyte lineages in the blood and other organs was determined by staining for specific T or B cell markers by flow cytometry analysis at different timepoints.

Statistical Analysis

Data from the α4 null and control chimeric mice were analyzed and compared for statistically significant differences using the Student's t test.

Acknowledgments

Correspondence should be addressed to R. O. H. We would like to thank Marge Cummiskey for her help in generating chimeric mice and Kim Mercer for the histological preparations. We greatly appreciate the invaluable help of Glenn Paradis from the flow cytometry facility. Thanks to Juan Lafaille for his constant help and support, to Hidde Ploegh and Jianzhu Chen for their interesting suggestions and critical reading of the manuscript, and to Norbert Wagner for communicating data prior to publication. Thanks to Begoña Galocha, José A. Villadangos, Daniela Taverna, and Herbert Haack for their helpful comments on the manuscript. This work has been supported by European Molecular Biology Organization and Human Frontier Science Program fellowships to A. G. A. (ALTF-583-1994 and LT-20/95), by a grant from the National Heart, Lung, and Blood Institute (POI HL41484), and by the Howard Hughes Medical Institute. J. T. Y. was an Associate and R. O. H. is an Investigator of the Howard Hughes Medical Institute.

Received March 17, 1996; revised May 6, 1996.

References

Altevogt, P., Hubbe, M., Ruppert, M., Lohr, J., von Hoegen, P., Sammar, M., Andrew, D.P., McEvoy, L., Humphries, M.J., and Butcher, E.C. (1995). The α4 integrin chain is a ligand for α4β7 and α4β1. J. Exp. Med. *182*, 345–355.

Boismenu, R., Rhein, M., Fischer, W.H., and Havran, W.L. (1996). A role for CD81 in early T cell development. Science *271*, 198–200.

Boudreau, N., Sympson, C.J., Werb, Z., and Bissell, M.J. (1995). Suppression of ICE and apoptosis in mammary epithelial cells by extracellular matrix. Science *267*, 891–893.

Chen, J., Lansford, R., Stewart, V., Young, F., and Alt, F.W. (1993). RAG-2-deficient blastocyst complementation: an assay of gene function in lymphocyte development. Proc. Natl. Acad. Sci. USA *90*, 4528–4532.

Cheng, A.M., Rowley, B., Pao, W., Hayday, A., Bolen, J.B., and Pawson, T. (1995). Syk tyrosine kinase required for mouse viability and B-cell development. Nature *378*, 303–306.

Dorshkind, K. (1990). Regulation of hemopoiesis by bone marrow stromal cells and their products. Annu. Rev. Immunol. *8*, 111–137.

George, E.L., and Hynes, R.O. (1994). Gene targeting and generation of mutant mice for studies of cell-extracellular matrix interactions. Meth. Enzymol. *245*, 386–420.

Gisler, R.H., Hollander, G., and Soderberg, A. (1987). Functional maturation of murine B lymphocyte precursors. I. Selection of adherent cell-dependent precursors from bone marrow and fetal liver. J. Immunol. *138*, 2427–2432.

Gordon, M.Y., Dowding, C.R., Riley, G.P., Goldman, J.M., and Greaves, M.F. (1987). Altered adhesive interactions with marrow stroma of haematopoietic progenitor cells in chronic myeloid leukaemia. Nature *328*, 342–344.

Hamann, A., Andrew, D.P., Jablonski-Westrich, D., Holzmann, B., and Butcher, E.C. (1994). Role of α4-integrins in lymphocyte homing to mucosal tissues *in vivo*. J. Immunol. *152*, 3282–3293.

Hardin, J.A., Yamaguchi, K., and Sherr, D.H. (1995). The role of peritoneal stromal cells in the survival of $sIgM^+$ peritoneal B lymphocyte population. Cell. Immunol. *161*, 50–60.

Hardy, R.R., Carmack, C.E., Shinton, S.A., Kemp, J.D., and Hayakawa, K. (1991). Resolution and characterization of pro-B and pre-pro-B cell stages in normal mouse bone marrow. J. Exp. Med. *173*, 1213–1225.

Hemler, M.E. (1990). VLA proteins in the integrin family: structures, functions and their role in leukocytes. Annu. Rev. Immunol. *8*, 365–400.

Hirsch, E., Iglesias, A., Potocnik, A.J., Hartmann, U., and Fassler, R. (1996). Impaired migration but not differentiation of haematopoietic stem cells in the absence of β1 integrins. Nature *380*, 171–175.

Holzmann, B., and Weissman, I.L. (1989). Peyer's patch-specific lymphocyte homing receptors consist of a VLA-4 like α chain associated with either of two integrin β chains, one of which is novel. EMBO J. *8*, 1735–1741.

Hynes, R.O. (1992). Integrins: versatility, modulation, and signaling in cell adhesion. Cell *69*, 11–25.

Jarvis, L.J., and LeBien, T.W. (1995). Stimulation of human bone marrow stromal cells tyrosine kinases and IL-6 production by contact with B lymphocytes. J. Immunol. *155*, 2359–2368.

Jotereau, F., Heuze, F., Salomon-Vie, V., and Gascan, H. (1987). Cell kinetics in the fetal mouse thymus: precursor cell input, proliferation, and emigration. J. Immunol. *138*, 1026–1030.

Kantor, A.B., and Herzenberg, L.A. (1993). Origin of murine B cell lineages. Annu. Rev. Immunol. *11*, 501–538.

Kerst, J.M., Sanders, J.B., Slaper-Cortenbach, I.C.M., Doorakkers, M.Ch., Hooibrink, B., van Oers, R.H.J., von dem Borne, A.E.G.Kr., and van der Schoot, C.E. (1993). α4β1 and α5β1 are differentially expressed during myelopoiesis and mediate the adherence of human $CD34^+$ cells to fibronectin in an activation-dependent way. Blood *81*, 344–351.

Kincade, P.W., Lee, G., Pietrangeli, C.E., Hayashi, S.-I., and Gimble, J.M. (1989). Cells and molecules that regulate B lymphopoiesis in bone marrow. Annu. Rev. Immunol. *7*, 111–143.

Koopman, G., Keehnen, R.M.J., Lindhout, E., Newman, W., Shimizu, Y., van Seventer, G.A., de Groot, C., and Pals, S.T. (1994). Adhesion through the LFA-1 (CD11a/CD18)-ICAM-1 (CD54) and the VLA-4 (CD49d)-VCAM-1 (CD106) pathways prevents apoptosis of germinal center B cells. J. Immunol. *152*, 3760–3767.

Lafaille, J.J., DeCloux, A., Bonneville, M., Takagaki, Y., and Tonegawa, S. (1989). Junctional sequences of T cell receptor γδ genes: implications for γδ T cell lineages and for a novel intermediate of V-(D)-J joining. Cell *59*, 859–870.

Ledbetter, J., Goding, J., Tsu, T., and Herzenberg, L.A. (1979). A new mouse lymphoid sialoantigen (Lgp-100) recognized by a monoclonal rat antibody. Immunogenetics *8*, 347–360.

Lee, M.-G., Sharrow, S.O., Farr, A.G., Singer, A., and Udey, M.C. (1994). Expression of the homotypic adhesion molecule E-cadherin by immature murine thymocytes and thymic epithelial cells. J. Immunol. *152*, 5653–5659.

Levesque, J.P., Leavesley, D.I., Niutta, S., Vadas, M., and Simmons, P.J. (1995). Cytokines increase human hematopoietic cell adhesiveness by activation of very late antigen (VLA)-4 and VLA-5 integrins. J. Exp. Med. *181*, 1805–1815.

Li, Y.-S., Hayakawa, K., and Hardy, R.R. (1993). The regulated expression of B lineage associated genes during B cell differentiation in bone marrow and fetal liver. J. Exp. Med. *178*, 951–960.

Merville, P., Dechanet, J., Desmouliere, A., Durand, I., de Bouteiller, O., Garrone, P., Banchereau, J., and Liu, Y.-J. (1996). Bcl-2^+ tonsillar plasma cells are rescued from apoptosis by bone marrow fibroblasts. J. Exp. Med. *183*, 227–236.

Miyake, K., Weissman, I.L., Greenberger, J.S., and Kincade, P.W. (1991a). Evidence for a role of the integrin VLA-4 in lympho-hemopoiesis. J. Exp. Med. *173*, 599–607.

Miyake, K., Medina, K., Ishihara, K., Kimoto, M., Auerbach, R., and Kincade, P.W. (1991b). A VCAM-like adhesion molecule on murine bone marrow stromal cells mediates binding of lymphocyte precursors in culture. J. Cell Biol. *114*, 557–565.

Miyake, K., Hasunuma, Y., Yagita, H., and Kimoto, M. (1992). Requirement for VLA-4 and VLA-5 integrins in lymphoma cells binding to and migration beneath stromal cells in culture. J. Cell Biol. *119*, 653–662.

Mombaerts, P., Iacomini, J., Johnson, R.S., Herrup, K., Tonegawa, S., and Papaioannou, V.E. (1992). RAG-1-deficient mice have no mature B and T lymphocytes. Cell *68*, 869–877.

Morrison, S.J., Uchida, N., and Weissman, I.L. (1995). The biology of hematopoietic stem cells. Annu. Rev. Cell. Dev. Biol. *11*, 35–71.

Mortensen, R.M., Conner, D.A., Chao, S., Geisterfer-Lowrance, A.A.T., and Seidman, J.G. (1992). Production of homozygous mutant ES cells with a single targeting construct. Mol. Cell. Biol. *12*, 2391–2395.

Motoyama, N., Wang, F., Roth, K., Sawa, H., Nakayama, K.-i., Nakayama, K., Negishi, I., Senju, S., Zhang, Q., Fujii, S., and Loh, D.Y. (1995). Massive cell death of immature hematopoietic cells and neurons in Bcl-x-deficient mice. Science *267*, 1506–1510.

Nakayama, K.-i., Nakayama, K., Negishi, I., Kuida, K., Shinkai, Y., Louie, M.C., Fields, L.E., Lucas, P.J., Stewart, V., Alt, F.W., and Loh, D.Y. (1993). Disappearance of the lymphoid system in Bcl-2 homozygous mutant chimeric mice. Science *261*, 1584–1588.

Nosaka, T., van Deursen, J.M.A., Tripp, R.A., Thierfelder, W.E., Witthuhn, B.A., McMickle, A.P., Doherty, P.C., Grosveld, G.C., and Ihle, J.N. (1995). Defective lymphoid development in mice lacking Jak3. Science *270*, 800–802.

Papayannopoulou, T., and Nakamoto, B. (1993). Peripheralization of hemopoietic progenitors in primates treated with anti-VLA4 integrin. Proc. Natl. Acad. Sci. USA *90*, 9374–9378.

Papayannopoulou, T., Craddock, C., Nakamoto, B., Priestley, G.V., and Wolf, N.S. (1995). The VLA4/VCAM-1 adhesion pathway defines contrasting mechanisms of lodgment of transplanted murine hemopoietic progenitors between bone marrow and spleen. Proc. Natl. Acad. Sci. USA *92*, 9647–9651.

Qian, F., Vaux, D.L., and Weissman, I.L. (1994). Expression of the integrin α4β1 on melanoma cells can inhibit the invasive stage of metastasis formation. Cell *77*, 335–347.

Roldán, E., García-Pardo, A., and Brieva, J.A. (1992). VLA-4-fibronectin interaction is required for the terminal differentiation of human bone marrow cells capable of spontaneous and high rate immunoglobulin secretion. J. Exp. Med. *175*, 1739–1747.

Ruiz, P., Wiles, M.V., and Imhof, B.A. (1995). α6 integrins participate in pro-T cell homing to the thymus. Eur. J. Immunol. *25*, 2034–2041.

Ruoslahti, E., and Reed, J.C. (1994). Anchorage dependence, integrins, and apoptosis. Cell *77*, 477–478.

Russell, S.M., Tayebi, N., Nakajima, H., Riedy, M.C., Roberts, J.L., Aman, M.J., Migone, T.-S., Noguchi, M., Markert, M.L., Buckley, R.H., O'Shea, J.J., and Leonard, W.J. (1995). Mutation of Jak3 in a patient with SCID: essential role of Jak3 in lymphoid development. Science *270*, 797–800.

Salomon, D.R., Mojcik, C.F., Chang, A.C., Wadsworth, S., Adams, D.H., Coligan, J.E., and Shevach, E.M. (1994). Constitutive activation of integrin α4β1 defines a unique stage of human thymocyte development. J. Exp. Med. *179*, 1573–1584.

Sánchez-Madrid, F., and Corbí, A.L. (1992). Leukocyte integrins: structure, function and regulation of their activity. Semin. Cell Biol. *8*, 1735–1741.

Sawada, M., Nagamine, J., Takeda, K., Utsumi, K., Kosugi, A., Tatsumi, Y., Hamaoka, T., Miyake, K., Nakajima, K., Watanabe, T., Sakakibara, S., and Fujiwara, H. (1992). Expression of VLA-4 on thymocytes: maturation stage-associated transition and its correlation with their capacity to adhere to thymic stromal cells. J. Immunol. *149*, 3517–3524.

Schwartzberg, P.L., Stall, A.M., Hardin, J.D., Bowdish, K.S., Humaran, T., Boast, S., Harbison, M.L., Robertson, E.J., and Goff, S.P. (1991). Mice homozygous for the *abl*^{m1} mutation show poor viability and depletion of selected B and T cell populations. Cell *65*, 1165–1175.

Scollay, R., Smith, J., and Stauffer, V. (1986). Dynamics of early T cells: prothymocyte migration and proliferation in the adult mouse thymus. Immunol. Rev. *91*, 129–157.

Sheppard, A.M., Onken, M.D., Rosen, G.D., Noakes, P.G., and Dean, D.C. (1994). Expanding roles for α4 integrin and its ligands in development. Cell Adhes. Comm. *2*, 27–43.

Shinkai, Y., Rathbun, G., Lam, K.-P., Oltz, E.M., Stewart, V., Mendelsohn, M., Charron, J., Datta, M., Young, F., Stall, A.M., and Alt, F.W. (1992). RAG-2-deficient mice lack mature lymphocytes owing to inability to initiate V(D)J rearrangement. Cell *68*, 855–867.

Simmons, P.J., Masinovski, B., Longenecker, B.M., Berenson, R., Torok-Storb, B., and Gallatin, W.M. (1992). Vascular cell adhesion molecule-1 expressed by bone marrow stromal cells mediates the binding of hematopoietic progenitor cells. Blood *80*, 388–395.

Springer, T.A. (1994). Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. Cell *76*, 301–314.

Strasser, A., Rolink, A., and Melchers, F. (1989). One synchronous wave of B cell development in mouse fetal liver changes at day 16 of gestation from dependence to independence of a stromal cell environment. J. Exp. Med. *170*, 1973–1986.

Sudo, T., Ito, M., Ogawa, Y., Iizuka, M., Kodama, H., Kunisada, T., Hayashi, S.-I., Ogawa, M., Sakai, K., Nishikawa, S., and Nishikawa, S.-I. (1989). Interleukin 7 production and function in stromal cell-dependent B cell development. J. Exp. Med. *170*, 333–338.

Teixidó, J., Hemler, M.E., Greenberger, J.S., and Anklesaria, P. (1992). Role of β1 and β2 integrins in adhesion of human $CD34^{hi}$ stem cells to bone marrow stroma. J. Clin. Invest. *90*, 358–367.

Thomis, D.C., Gurniak, C.B., Tivol, E., Sharpe, A.H., and Berg, L.J. (1995). Defects in B lymphocyte maturation and T lymphocyte activation in mice lacking Jak3. Science *270*, 794–797.

Turner, M., Mee, P.J., Costello, P.S., Williams, O., Price, A.A., Duddy, L.P., Furlong, M.T., Geahlen, R.L., and Tybulewicz, V.L.J. (1995). Perinatal lethality and blocked B-cell development in mice lacking the tyrosine kinase Syk. Nature *378*, 298–302.

Tybulewicz, V.L.J., Crawford, C.E., Jackson, P.K., Bronson, R.T., and Mulligan, R.C. (1991). Neonatal lethality and lymphopenia in mice with a homozygous disruption of the c-*abl* proto-oncogene. Cell *65*, 1153–1163.

Utsumi, K., Sawada, M., Narumiya, S., Nagamine, J., Sakata, T., Iwagami, S., Kita, Y., Teraoka, H., Hirano, H., Ogata, M., Hamaoka, T., and Fujiwara, H. (1991). Adhesion of immature thymocytes to thymic stromal cells through fibronectin molecules and its significance for the induction of thymocyte differentiation. Proc. Natl. Acad. Sci. USA *88*, 5685–5689.

Veis, D.J., Sorenson, C.M., Shutter, J.R., and Korsmeyer, S.J. (1993). Bcl-2-deficient mice demonstrate fulminant lymphoid apoptosis, polycystic kidneys, and hypopigmented hair. Cell *75*, 229–240.

Weissman, I.L. (1994). Developmental switches in the immune system. Cell *76*, 207–218.

Williams, D.A., Rios, M., Stephens, C., and Patel, V.P. (1991). Fibronectin and VLA-4 in haematopoietic stem cell-microenvironment interactions. Nature *352*, 438–441.

Yang, J.T., Rayburn, H., and Hynes, R.O. (1995). Cell adhesion events mediated by α4 integrins are essential in placental and cardiac development. Development *121*, 549–560.

Yednock, T.A., Cannon, C., Fritz, L.C., Sanchez-Madrid, F., Steinman, L., and Karin, N. (1992). Prevention of experimental autoimmune encephalomyelitis by antibodies against α4β1 integrin. Nature *356*, 63–66.